Evaluation of a Commercial Immunoenzymometric Assay for Alpha-Fetoprotein

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A two-site immunoenzymometric assay (Abbott Diagnostics) for alpha-fetoprotein (AFP) in maternal serum and amniotic fluid has been evaluated for its suitability as a screening test for open neural tube defects. In a retrospective study based on 190 pregnancies of known outcome, performance of the kit in measuring both serum and amniotic fluid AFP correlated well with that of an in-house radiomunoassay. Of 39 pregnancies associated with open neural tube defects, only four would not have been detected by the use of sequential measurement of serum and amniotic fluid AFP (also essentially in agreement with results obtained by the RIA). We conclude that this immunoassay could form the basis for a screening program for antenatal detection of open neural tube defects.

Additional Keyphrases: fetal status • amniotic fluid

Measurement of alpha-fetoprotein (AFP) concentrations in maternal serum in the second trimester of pregnancy is used widely to screen for open neural tube defects (NTD) in the fetus, a practice that has led to a significant decrease in the number of babies born with this condition (1). Where centralized laboratory testing is not available, diagnosis depends on the availability of reliable commercial kits. Clearly, such kits must perform accurately and reproducibly, the more so where individual workloads are small, so that fewer data are generated for deriving reference values. Most procedures used so far have been conventional radioimmunoassays. Recently, several two-site assays for AFP have been described, involving the use of antibodies with non-isotopic labels (2, 3). These methods offer the potential of improved speed, sensitivity, and dose-response range, as compared with competitive immunoassays. We report here an evaluation of a commercial "two-site" kit in which an enzyme is the non-isotopic label.

Materials and Methods

Immunoassay. The kit evaluated was the Abbott AFP-EIA (Abbott Diagnostics, D-6200 Wiesbaden-Delkenheim, F.R.G.). The kit's two-site procedure is based on goat antibody labeled with horseradish peroxidase (EC 1.11.1.7) and goat antibody immobilized on single beads. The enzyme label is assayed by oxidation of o-phenylenediamine, the resulting orange color being monitored at 492 nm with the Abbott Quantum photometer. The assay procedure was carried out according to the manufacturer's instructions. The in-house procedure used for comparison was a conventional double-antibody radiomunoassay. The entire procedure, including data reduction, was performed by the Kemtek 3000 Automated Immunoassay System (Kemble Instruments, Burgess Hill, Sussex, U.K.) (4).

Patients' samples. Amniotic fluids, obtained by amniocentesis during the middle trimester of pregnancy, were centrifuged to remove any cells; we assayed aliquots of the supernates for AFP. The remaining supernates were then stored at −20 °C for as long as six years. Serum samples were also taken from all patients either at the time of amniocentesis or within the preceding two weeks, and were stored at −20 °C after their initial analysis.

The outcome of all pregnancies studied was known. Where possible (80% of cases) the gestational age at the time of amniocentesis and venepuncture was determined by ultrasound scanning. If this information was not available, the gestational age was estimated from the date of the last menstrual period.

Median AFP values at each week of gestation for the kit procedure were based on the analysis of 200 randomly selected samples, which were being tested as part of the routine screening program for NTD.

To avoid any possible effects of storage on measured AFP concentrations, we re-assayed all of the samples by the RIA at the same time that we assayed them with the kit.

Results

We based the performance data on five sequential runs of 125 patients' samples in duplicate. The precision profile for the AFP-EIA kit, derived according to the method of Ekins (5), demonstrated a coefficient of variation <10% for AFP concentrations in serum of 25 to 400 µg/L.

As shown in Figure 1, there was a good correlation between AFP concentrations measured with AFP-EIA (y) and RIA (x). For serum the equation for the linear regression was y = 1.11x − 1.95 (r = 0.97). For amniotic fluid the equation was y = 1.03x + 0.69 (r = 0.95).

![Fig. 1. Comparison of results for serum AFP by both assays.](image-url)

Concentrations exceeding the highest-concentration standard of the in-house assay (280 µg/L) and less than the lowest standard (14 µg/L) are not shown; however, the corresponding results measured by AFP-EIA were also outside these limits. ⊙ = normal outcome, □ = closed spine bifida, △ = open spine bifida, ■ = anencephaly.
Figure 2 shows, for 39 cases of open NTD, the AFP concentrations measured by the kit in serum as a function of gestational age. Only two values fell below the recommended level for further action, 2.5 multiples of the median (M.o.M.) (6), being 2.2 and 2.4 M.o.M. The values obtained in the RIA on these two samples were borderline (2.6 and 2.7 M.o.M., respectively), just as they had been when originally assayed.

In two amniotic fluids from pregnancies associated with open spina bifida, AFP concentrations measured by the AFP-EIA were below the intervention level recommended by the U.K. collaborative study (7), being 2.9 M.o.M. at 16 weeks and 2.2 M.o.M. at 20 weeks' gestation. These samples also gave falsely low values by the RIA (2.7 and 2.0 M.o.M., respectively), as did one other (2.3 M.o.M. at 18 weeks' gestation) that was correctly assigned by the EIA (4.0 M.o.M.). There were no false-positive results for amniotic fluids by either method (Figure 2, right).

Discussion

Essential requirements for a new method for the measurement of AFP are that it should be accurate and reproducible. This evaluation has shown that these requirements can be met by the Abbott AFP-EIA. Results obtained with the assay correlated well with those obtained by an in-house method that has for several years been the basis of a successful screening program for NTD. There were no markedly discrepant results, even at high concentrations of AFP. The slight positive bias relative to our in-house method is not in itself a problem and merely serves to underline the importance of establishing individual laboratory reference ranges. At the concentrations of AFP in maternal serum at which further investigation is considered warranted (60–120 μg/L, depending on gestational age), the between-assay CV for the kit was less than 6%, which compared favorably with that of the automated RIA procedure.

Given its satisfactory performance, the success of this kit depends on its convenience. One of its major advantages is that it can provide results for 50 patients' samples within a day. However, the assay is somewhat labor intensive and the Abbott Quantum photometer requires handling tubes individually. Laboratories adopting a large workload might therefore require a higher level of automation.

The demonstration that the kit is capable of identifying known cases of open NTD from measurements of serum and amniotic fluid AFP suggests that it is as reliable diagnostically as existing assay methods. Two cases of anencephaly would have been missed by the kit on the basis of their AFP concentrations in serum (none missed by RIA) and two cases of open spina bifida on the basis of their AFP concentrations in amniotic fluid (three missed by RIA). Like the RIA, the AFP-EIA gave no false-positive results for amniotic fluids.

Finally, we note that this study could not address the problem of the long-term stability of performance of the immunoenzymetric assay. Although the use of an enzyme label should alleviate problems in this area, laboratories using the kit will have to practice reliable internal quality-assessment procedures to detect any changes in assay performance with the purchase of different kit lots.

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Liquid-Chromatographic Assay of Dexamethasone in Plasma

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We report a simple, efficient method for removing dexamethasone from plasma by use of solid-phase extraction columns. The dexamethasone is then quantified by "high-performance" liquid chromatography with ultraviolet detection at 254 nm. Dexamethasone concentrations and peak-height ratios were linearly related over the range 5 to 200 µg/L. The lower limit of sensitivity was 5 µg/L. The average recoveries of dexamethasone and methylprednisolone (internal standard) were 72 and 71%, respectively. This procedure offers improved efficiency over that of previously described methods by decreasing analytical time and improving sample cleanup. Sensitive, specific, and relatively inexpensive, this method is suitable for most clinical applications.

Additional Keyphrases: sample preparation • corticosteroids • methylprednisolone as internal standard

Dexamethasone (9-fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione) is a synthetic corticosteroid that is important in treating many allergic and inflammatory conditions (1,2). In moderate to large doses, dexamethasone is also an effective anti-emetic in the treatment of chemotherapy-induced nausea and vomiting in cancer patients (3-5). High doses are routinely used, but the therapeutic plasma concentrations for this indication have not been defined.

Radioimmunoassay procedures, the most commonly used techniques for determining dexamethasone in biological fluids, require the time-consuming and expensive preparation of a specific antibody (6-8). Recently, several investigators have described methods for dexamethasone analysis in human plasma in which normal or reversed-phase "high-pressure" liquid chromatography (HPLC) is used (9-10). These procedures are indeed specific and sensitive but are complicated and time-consuming, requiring between two and five extraction steps before analysis. Here we report a reversed-phase HPLC method with ultraviolet detection in which the dexamethasone is extracted from plasma on solid-phase extraction columns.

Materials and Methods

Reagents. Dexamethasone, methylprednisolone, and triethylamine were obtained from Sigma Chemical Company, St. Louis, MO, and used as received. Acetonitrile for use in the HPLC separation was "distilled-in-glass" grade, from MCB Reagents, Cincinnati, OH 45212. Chloroform (from MCB) and methanol (from Burdick and Jackson Laboratories, Inc., Muskegon, MI) for use in the extraction procedure were analytical grade. Filtered de-ionized water was supplied by a water-purification system (Corning Glass Works, Corning, NY). Silica and C₁₈ Bond-Elut extraction columns (both 1-ml capacity) were from Analytichem International, Harbor City, CA.

Apparatus. A Model 210 sample-injection valve and a Model 112 solvent-delivery module (both from Beckman Instruments, Fullerton, CA 92634) were used with the HPLC to monitor the column effluent. We used a Beckman Model 160 absorbance detector set at 254 nm and a sensitivity of 0.01 A full scale. Peak heights were measured and recorded with a Model 3390A reporting integrator (Hewlett Packard, Avondale, PA 19311). The 160 × 4.6 mm column was prepacked with "ultrasphere" octadecylsilane, of 5 µm average particle diameter (Altex, Berkeley, CA).

Procedures. The HPLC solvent was prepared by mixing 280 mL of acetonitrile with 720 mL of de-ionized water. We then added 200 µL of triethylamine to each liter of solution. The final mixture was passed through a 0.5-µm (pore-size) filter (Millipore Corp., Bedford, MA), then degassed by sonicating under reduced pressure for 10 min. A flow rate of 1.3 mL/min at 1.9 kPa was maintained. Chart speed was 20 cm/h. All procedures were carried out at room temperature.

Silica extraction columns were connected in series above the C₁₈ columns with a fitted Bond-Elut adaptor. On the day before the analysis, each set of columns was washed with 2 mL of methanol followed by 2 mL of de-ionized water. On the day of the procedure, the columns were prepared by washing with 3 mL of methanol, then with 4 mL of de-ionized water. To each sample and standard, we added 76 ng of methylprednisolone (internal standard) in 38 µL of water. We aspirated fluids and samples through the columns under reduced pressure, using a 10-place vacuum manifold (VacElut; Analytichem International). The samples consisted of 0.5 to 2.0 mL of plasma, applied after dilution with de-